

Application Ser. No. 10/729,576
Rule 116 Amendment and Response

Attorney Docket No.: 60409CON(50370)

AMENDMENTS TO THE CLAIMS

Please amend claims 1, 6, 8-10, 14, 22, 26, 35 and 36 and please cancel claims 7, 11-13, 21 and 39-42 without prejudice or disclaimer. The following listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Currently Amended) A method for identifying a test compound that modulates a heterologous receptor in a cell, said method comprising: providing a cell which comprises a heterologous receptor that is functionally integrated into a signal transduction pathway of said cell, wherein cell surface presentation of a detectable signal comprising a protein product of the AGA2 gene is induced upon activation of said signal transduction pathway; contacting said cell with a test compound; and detecting the level of expression of said detectable signal as a measure of the ability of said compound to modulate signaling via said heterologous receptor, whercin said detecting comprises: incubating said cell with a Sag1 protein conjugated with a reporter moiety, wherein said Sag1 protein binds specifically to said detectable signal; washing said cell to remove unbound Sag1 protein; and measuring the readout from said reporter moiety.

2. (Original) The method of claim 1, whercin said cell is a yeast cell.

3. (Original) The method of claim 2, whercin said signal transduction pathway is a yeast pheromone response pathway.

4. (Original) The method of claim 3, wherein said cell is a MAT α *Saccharomyces cerevisiae* cell.

5. (Cancelled)

6. (Currently Amended) The method of claim 1, wherein said detection step further ~~comprises: incubating said cell with a detector molecule conjugated with a reporter moiety; wherein said detector molecule binds specifically to said detectable signal; washing said cell to remove unbound detector molecules; incubating said cell with a substrate appropriate for said reporter moiety; and measuring the readout from said reporter moiety.~~

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7. (Cancelled)

8. (Currently Amended) The method of claim 7~~1~~, wherein said Sag1 protein comprises amino acids 20-352 of the mature protein.

9. (Currently Amended) The method of claim 6, wherein said reporter moiety is a reporter gene.

10. (Currently Amended) The method of claim 6, wherein said reporter gene encodes a polypeptide selected from the group consisting of beta-lactamase, peroxidase, luciferase, and alkaline phosphatase.

Claims 11 - 13 (Cancelled)

14. (Currently Amended) The method of claim 1~~1~~, wherein said reporter moiety is a fluorophore.

15. (Original) The method of claim 12, wherein said readout measuring step comprises a fluorescence polarization technique.

16. (Original) The method of claim 1, additionally comprising an extraction step, wherein said cell-surface expressed detectable signal is extracted from the cell prior to said detection step.

17. (Original) The method of claim 16, wherein said extraction step comprises treatment of said cell with a reducing agent.

18. (Original) The method of claim 17, wherein said detection step comprises: binding of said extracted detectable signal to a support; incubating said support with a detection molecule conjugated with a reporter moiety; and measuring the readout from said reporter moiety.

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19. (Previously presented) The method of claim 18, wherein said support comprises streptavidin-coated scintillation proximity assay beads containing scintillant.

20. (Original) The method of claim 19, wherein binding of said extracted detectable signal to said support is mediated by a biotinylated antibody, wherein said antibody binds specifically to said extracted detectable signal and also to said streptavidin-coated bead.

21. (Cancelled)

22. (Currently Amended) The method of claim ~~24~~18, wherein said Sag1 protein comprises amino acids 20-352 of the mature protein.

23. (Original) The method of claim 18, wherein said reporter moiety is a radiolabel.

24. (Original) The method of claim 23, wherein said radiolabel is ^{125}I or ^3H .

25. (Original) The method of claim 18, wherein said readout measuring step comprises detection of emitted light.

26. (Currently Amended) The method of claim 4, for identifying a test compound that modulates a heterologous receptor in a MAT α *Saccharomyces cerevisiae* cell, said method comprising: providing a MAT α *Saccharomyces cerevisiae* cell which comprises a heterologous receptor that is functionally integrated into a pheromone response pathway of said cell, wherein cell surface presentation of a detectable signal comprising a protein product of the AGA2 gene is induced upon activation of said pheromone response pathway and wherein said *S. cerevisiae* cell has the endogenous AGA1 gene deleted, such that the AGA2 gene product is secreted; contacting said cell with a test compound; and detecting the level of expression of said detectable signal as a measure of the ability of said compound to modulate signaling via said heterologous receptor.

27. (Original) The method of claim 26, wherein said detection step comprises: binding of said secreted AGA2 gene product to a support; incubating said support with a detection

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molecule conjugated with a reporter moiety; and measuring the readout from said reporter moiety.

28. (Original) The method of claim 27, wherein said support comprises streptavidin-coated SPA beads containing scintillant.

29. (Original) The method of claim 28, wherein binding of said secreted Aga2 protein to said support is mediated by a biotinylated antibody, wherein said antibody binds specifically to the secreted Aga2 protein and also to said streptavidin-coated bead.

30. (Original) The method of claim 27, wherein said detection molecule is the Sag1 protein.

31. (Original) The method of claim 30, wherein said Sag1 protein comprises amino acids 20-352 of the mature protein.

32. (Original) The method of claim 27, wherein said reporter moiety is a radiolabel.

33. (Original) The method of claim 32, wherein said radiolabel is ^{125}I or ^3H .

34. (Original) The method of claim 27, wherein said readout detection step comprises detection of emitted light.

35. (Currently Amended) The method of claim 2 or 26, wherein said heterologous receptor is a G-protein coupled receptor.

36. (Currently Amended) The method of claim 2 or 26, wherein said heterologous receptor is selected from the group consisting of melatonin receptor 1a, galanin receptor 1, neurotensin receptor, adenosine receptor 2a, somatostatin receptor 2, and corticotropin releasing factor receptor 1.

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37. (Original) The method of claim 36, wherein said heterologous receptor is melatonin receptor 1a.

38. (Original) The method of claim 35, wherein said heterologous G-protein coupled receptor functionally couples to the endogenous yeast GPA-1 protein subunit.

Claims 39-42 (Cancelled)